

FORM PTO-1390 (Modified)
(REV. 11-98)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES

2727-133

DESIGNATED/ELECTED OFFICE (DO/EO/US)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)

CONCERNING A FILING UNDER 35 U.S.C. 371

09/786011

INTERNATIONAL APPLICATION NO.

PCT/EP99/06453

INTERNATIONAL FILING DATE

2 September 1999 (02.09.99)

PRIORITY DATE CLAIMED

2 September 1998 (02.09.98)

TITLE OF INVENTION

"Oligonucleotides, Method and Kit for Detecting Listeria Monocytogenes by Nucleic Acid Amplification and/or Nucleic Acid Hybridization"

APPLICANT(S) FOR DO/EO/US

Pia Scheu, Alexander Gasch and Kornelia Berghof

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Declaration by inventors (unsigned)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5) 09/786011	INTERNATIONAL APPLICATION NO. PCT/EP99/06453	ATTORNEY'S DOCKET NUMBER 2727-133
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21. The following fees are submitted:				CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :					
<input type="checkbox"/>	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO		\$970.00		
<input checked="" type="checkbox"/>	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO		\$840.00		
<input type="checkbox"/>	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO		\$690.00		
<input type="checkbox"/>	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)		\$670.00		
<input type="checkbox"/>	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)		\$96.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$860.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30				\$0.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	13 - 20 =	0	x \$18.00	\$0.00	
Independent claims	2 - 3 =	0	x \$78.00	\$0.00	
Multiple Dependent Claims (check if applicable).			<input type="checkbox"/>	\$0.00	
TOTAL OF ABOVE CALCULATIONS =				\$860.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable).			<input checked="" type="checkbox"/>	\$430.00	
SUBTOTAL =				\$430.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30			+	\$0.00	
TOTAL NATIONAL FEE =				\$430.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).			<input type="checkbox"/>	\$0.00	
TOTAL FEES ENCLOSED =				\$430.00	
				Amount to be:	\$
				refunded	
				charged	\$

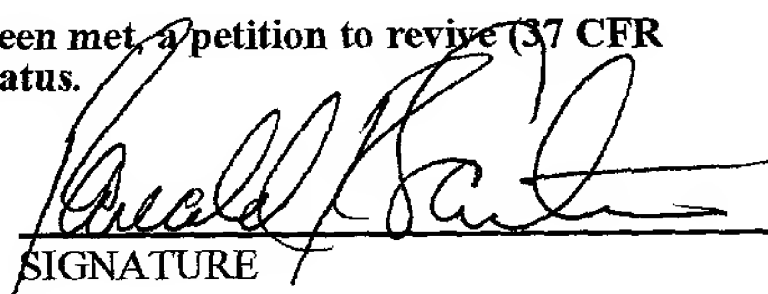
- ☒ A check in the amount of **\$430.00** to cover the above fees is enclosed.
- ☐ Please charge my Deposit Account in the amount of _____ to cover the above fees.
A duplicate copy of this sheet is enclosed.
- ☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **501145** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Ronald R. Santucci
Pitney, Hardin, Kipp & Szuch, LLP
711 Third Avenue, 20th Floor
New York, New York 10017

(212)687-6000


SIGNATURE
Ronald R. Santucci
NAME
28,988
REGISTRATION NUMBER
February 28, 2001
DATE

2727-133

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Pia Scheu, Alexander Gasch and Kornelia Berghof

Serial No.: Not Yet Assigned

International Appln. No.: PCT/EP99/06453

International Filing Date: 2 September 1999

Priority Date Claimed: 2 September 1998

For: OLIGONUCLEOTIDES, METHOD AND KIT FOR DETECTING LISTERIA
MONOCYTOGENES BY NUCLEIC ACID AMPLIFICATION AND/OR NUCLEIC ACID
HYBRIDIZATION

PRELIMINARY AMENDMENT

Box PCT
Commissioner for Patents
Washington, D.C. 20231
Attn: DO/EO/US

S I R:

Preliminary to examination of the above-identified
application kindly amend the application as follows:

In the Claims:

Kindly rewrite claims 4-9 and 12 as follows:

4. (Amended) Nucleic acid molecule according to claim 1,
characterized in that it is present in single-stranded or double-
stranded form.

5. (Amended) Nucleic acid molecule according to claim 1,
characterized in that it is present

- (i) as DNA sequence or
- (ii) as RNA sequence corresponding to (i) or
- (iii) as PNA sequence,

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where the nucleic acid molecule is modified, where appropriate, in a manner known per se for analytical detection methods, in particular for those based on hybridization and/or amplification.

6. (Amended) Nucleic acid molecule according to claim 1, characterized in that up to 20% of at least 10 successive nucleotides of its nucleotide chain, in particular 1 or 2 nucleotides, have been replaced by analogous building blocks known per se for probes and/or primers, in particular by nucleotides not naturally present in bacteria.

7. (Amended) Nucleic acid molecule according to claim 1, characterized in that the nucleic acid molecule has been modified or labeled by or additionally by having one or more radioactive groups, colored groups, fluorescent groups, groups for immobilization on a solid phase and/or groups for an indirect or direct reaction, in particular for an enzymatic reaction, in particular with the aid of antibodies, antigens, enzymes and/or substances with affinity to enzymes or enzyme complexes, and/or otherwise modifying or modified groups of a nucleic acid-like structure.

8. (Amended) Kit for analytical detection methods, in particular for detecting bacteria of the species *Listeria monocytogenes*, characterized by one or more nucleic acid molecules according to claim 1.

9. (Amended) Use of one or more of nucleic acid molecules according to claim 1 for detecting the presence of absence of bacteria of the species *Listeria monocytogenes*.

12. (Amended) Use according to claim 1, characterized in that the bacteria to be detected are distinguished from the bacteria not

to be detected on the basis of differences in the genomic DNA and/or RNA in at least one nucleotide position in the region of one of the nucleic acid molecules.

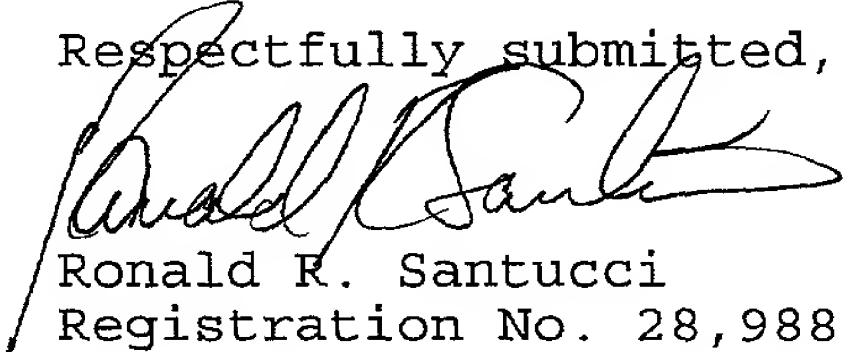
Kindly add new claim 13:

--13. (New) Use of a kit according to claim 8 for detecting the presence of absence of bacteria of the species *Listeria monocytogenes*.--

REMARKS

Initially, Applicants claim small entity status for this application. The claims of the above-identified application have been amended to remove all multiple dependencies. No new matter has been added. Accordingly, an early examination of the application is respectfully requested.

Respectfully submitted,


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APPENDIX:

4. (Amended) Nucleic acid molecule according to claim 1 [any of the preceding claims], characterized in that it is present in single-stranded or double-stranded form.

5. (Amended) Nucleic acid molecule according to claim 1 [any of the preceding claims], characterized in that it is present

- (i) as DNA sequence or
- (ii) as RNA sequence corresponding to (i) or
- (iii) as PNA sequence,

where the nucleic acid molecule is modified, where appropriate, in a manner known per se for analytical detection methods, in particular for those based on hybridization and/or amplification.

6. (Amended) Nucleic acid molecule according to claim 1 [any of the preceding claims], characterized in that up to 20% of at least 10 successive nucleotides of its nucleotide chain, in particular 1 or 2 nucleotides, have been replaced by analogous building blocks known per se for probes and/or primers, in particular by nucleotides not naturally present in bacteria.

7. (Amended) Nucleic acid molecule according to claim 1 [any of the preceding claims], characterized in that the nucleic acid molecule has been modified or labeled by or additionally by having one or more radioactive groups, colored groups, fluorescent groups, groups for immobilization on a solid phase and/or groups for an indirect or direct reaction, in particular for an enzymatic reaction, in particular with the aid of antibodies, antigens, enzymes and/or substances with affinity to enzymes or enzyme complexes, and/or otherwise modifying or modified groups of a nucleic acid-like structure.

8. (Amended) Kit for analytical detection methods, in particular for detecting bacteria of the species *Listeria monocytogenes*,

characterized by one or more nucleic acid molecules according to claim 1 [any of the preceding claims].

9. (Amended) Use of one or more [or] of nucleic acid molecules according to claim 1 [any of claims 1 to 7 or of a kit according to claim 8] for detecting the presence of absence of bacteria of the species *Listeria monocytogenes*.

12. (Amended) Use according to claim 1 [(sic)], characterized in that the bacteria to be detected are distinguished from the bacteria not to be detected on the basis of differences in the genomic DNA and/or RNA in at least one nucleotide position in the region of one of the nucleic acid molecules [according to claim 3].

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2727-133

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Pia Scheu, Alexander Gasch and Kornelia Beghof

Serial No.: 09/786,011

International Appln. No.: PCT/EP99/06453

International Filing Date: 02 September 1999

Priority Date Claimed: 02 September 1998

For: OLIGNUCLEOTIDES, METHOD AND KIT FOR DETECTING LISTERIA
MONOCYTOGENES BY NUCLEIC ACID AMPLIFICATION AND/OR
NUCLEIC ACID HYBRIDIZATION

SECOND PRELIMINARY AMENDMENT/FORWARDING OF SEQUENCE LISTING ON
DISK AND WRITTEN COPY

Box PCT
Commissioner for Patents
Washington, D.C. 20231
Attn: DO/EO/US

S I R:

In response to the Notification to Comply with Requirements
for Patent Applications Containing Nucleotide Sequence and/or
Amino Acid Sequence Disclosures, Applicants respectfully respond
as follows.

The Sequence Listing

Kindly include the attached Sequence Listing as part of the
application.

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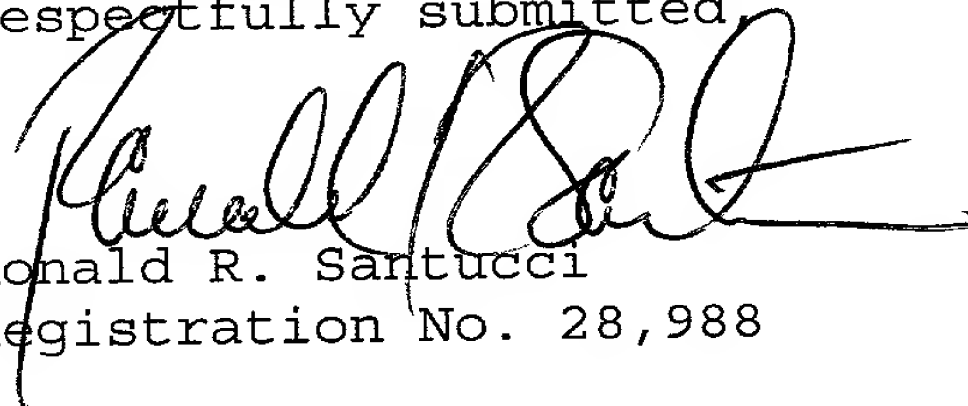
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Remarks

Applicants respectfully enclose herewith a substitute Sequence Listing both on a diskette and in written form for the above referenced application. The content of the computer readable copy is, upon information and belief, and to the best of my knowledge, the same as the content of the written sequence listing herewith provided is supported by the specification, and includes no new matter.

The Commissioner is authorized to charge any additional fees that may be required to Deposit Account No. 501145, Order No. 2727-133.

Respectfully submitted



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Oligonucleotides, method and kit for detecting *Listeria*
monocytogenes by nucleic acid amplification and/or
nucleic acid hybridization

- 5 The invention provides oligonucleotides, method and kit
for detecting *Listeria monocytogenes* by nucleic acid
amplification and/or nucleic acid hybridization.

The genus *Listeria* consists of the six species
10 *L. monocytogenes*, *L. grayi*, *L. innocua*, *L. ivanovii*,
L. seligeri and *L. welshimeri*. Among these, only
strains of the species *L. monocytogenes* are pathogenic
for humans, in particular for those with a weakened
immune system and for the elderly, and the newborn. The
15 most common symptoms of listeriosis are septicemia,
meningitis and miscarriages. *L. monocytogenes*
infections are caused especially by consuming
contaminated food, in particular milk products, meat,
poultry and vegetables.

20 A large number of methods for detecting
L. monocytogenes are known. Conventional detection
methods for *L. monocytogenes* comprise preconcentrating
and subsequently isolating colonies on selection media
25 (Lovett et al., J. Food Protection **50** (1987), 188-192;
McClain & Lee, J. Assoc. Off. Anal. Chem. **71** (1988),
660-664). Single colonies are examined for their
morphology or for biochemical or serological
properties. An analysis may take up to 6 - 8 days.

30 Since especially readily perishable food is frequently
contaminated with *L. monocytogenes*, various high-speed
methods for detecting *L. monocytogenes* have been
developed. Such methods are based either on
35 immunological methods or on the application of nucleic
acid probes.

In this connection, detection may be carried out by
direct hybridization of probes to microbe-specific DNA

or RNA (see, for example, Datta, A.R. et al., Appl. Environ. Microbiol. 53 (1987), 2256-2259). The disadvantage of those methods is the low sensitivity, since at least 10^5 - 10^6 copies of the target nucleic acid are required. This can be compensated by combination with an amplification of the target sequence, for example using the polymerase chain reaction (PCR). A plurality of PCR methods for detecting *L. monocytogenes* have been described in the literature [for a review see, for example, Jones, D.D. & Bej, A.K. in "PCR Technology, Current Innovations", Griffin, H.G & Griffin, A.M., eds., (1994), 341-365]. See also US patents 4,683,195; 4,683,202 and 4,965,188. Furthermore, the ligase chain reaction [WO publication 89/09835], "self-sustained sequence replication" [EP 329,822], "transcription based amplification system" [EP 310, 229] and Q β RNA replicase system [US patent 4,957,858] may be employed for the amplification of nucleic acids.

Some test kits for detection by means of antibodies are already commercially available. Most of these tests, however, display only low sensitivity and specificity.

To detect specific microorganisms by means of nucleic acid hybridization or nucleic acid amplification, microbe-specific oligonucleotides are commonly used whose base sequence is characteristic for the DNA or RNA of a specific microorganism or of a group of microorganisms. When using said microbe-specific oligonucleotides (for example as primers or probes) in connection with the methods mentioned above, hybridization to the DNA/RNA or amplification of DNA/RNA can occur under suitable reaction conditions only if the DNA/RNA of the particular microorganisms to be detected is present.

The detection methods described for *L. monocytogenes* are based mainly on those target genes which play a

role in the pathogenicity of *L. monocytogenes*. It is known that some of these genes are located on the chromosome next to each other in a virulence gene cluster. Since the listeriolysin gene (hlyA) has been
5 recognized first as to be clearly necessary for the pathogenicity of *L. monocytogenes* (Cossart, P. et al., Infect. Immun. **57** (1989), 3629-3636), most of the genotypic detection methods are based on this gene. The hlyA gene, however, is also found with high homology in
10 nonpathogenic listeria (i.e. in *L. seeligeri* and *L. ivanovii*). In said detection methods, the appearance of false-positive results cannot be completely dismissed, since single point mutations in the region of the binding sites of primers or probes may already be
15 sufficient for this.

It was possible to show that the metalloprotease gene (mpl) which is located in the genome right next to the hlyA gene is only present in *L. monocytogenes*, and thus
20 not in nonpathogenic listeria (Domann, E. et al., Infect. Immun. **59** (1991), 65-72).

The suitability in principle of the DNA region flanking the hlyA gene for detecting *L. monocytogenes* by means
25 of hybridization or amplification has been described (Rossen, L. et al., Int. J. Food Microbiol. **14** (1991), 145-152); however, no oligonucleotide sequences for such detection methods have been published yet.

30 The sequence of the *L. monocytogenes* mpl gene is described in the EMBL database under accession number X54619 [Domann, E. et al., Infect. Immun. **59** (1991), 65-72]. Furthermore, parts of the sequence of the *L. monocytogenes* mpl gene are listed in the EMBL
35 database under accession number X60035 [Rasmussen, O.F. et al., Infect. Immun. **59** (1991), 3945-3951].

It was an object of the present invention to develop a detection method which is suitable for routine use and

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in which the probability of false-positive results appearing is as low as possible for the particular user, even under very variable experimental conditions.

- 5 In particular, oligonucleotide sequences are to be provided which can be employed in a detection method for the *L. monocytogenes* metalloprotease gene (mpl).

10 These objects are achieved by providing nucleic acid molecules of the sequences

- (i) 5'-GAA AAA GCA TTT GAA GCC AT-3' or
(ii) 5'-GCA ACT TCC GGC TCA GC-3' or
(iii) 5'-TCG AAA AAG CAT TTG AAG CC-3' or
(iv) 5'-GGT CAG AGT GAA GCT CAT GT-3' or
(v) 5'-CTI TTC ACA TGA GCT TCA CTC TGA CCR A-3' or

(vi) 5'-CTT TTT CTT TCA CTG GGT TTC CGA CAT-3' or
(vii) 5'-GAT GAT TTC TTT TTC TTT CAC TGG ATT TCC AAT AT-3'
or

- 15 (viii) of the sequence complementary in each case to (i), (ii), (iii), (iv), (v), (vi), and (vii).

The oligonucleotides according to the invention may be defined as follows:

20

Oligonucleotide LM1: (sequence (i) = SEQ ID NO 1 corresponds to the position 2476 to 2495 of *L. monocytogenes* [according to Domann, E. et al. Infect. Immun. 59 (1991), 65-72).

25

Oligonucleotide LM 2: (sequence (ii) = SEQ ID NO 2) corresponds to the position 2608 to 2624 of *L. monocytogenes*.

Oligonucleotide LM 3: (sequence (iii) = SEQ ID NO 3) corresponds to the position 2474 to 2493 of

30

L. monocytogenes

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Oligonucleotide LM 4 (sequence (iv) = SEQ ID NO 4) corresponds to the position 2497 to 2516 of *L. monocytogenes*.

5 Oligonucleotide LMR 1: (sequence (v) = SEQ ID NO 5) corresponds to the position 2495 to 2522 of *L. monocytogenes*.

Oligonucleotide LMF 1: (sequence (vi) = SEQ ID NO 6) corresponds to the position 2525 to 2551 of *L. monocytogenes*.

10 Oligonucleotide LMF 2 (sequence (vii) = SEQ ID NO 7) corresponds to the position 2525 to 2559 of *L. monocytogenes*.

15 In order to investigate the extent to which sequence variations of the *mpl* gene occur within the species *L. monocytogenes*, an internal fragment of 300 base pairs of 13 *L. monocytogenes* strains of various serovars (2 strains of the serovars 1/2a, 1 strain of the serovar 1/2b, 1 strain of the serovar 1/2c, 1 strain of the serovar 3a, 1 strain of the serovar 3b, 1 strain of the serovar 3c, 1 strain of the serovar 4a, 1 strain of the serovar 4a/b, 1 strain of the serovar 4b, 1 strain of the serovar 4c, 1 strain of the serovar 4d, and 1 strain of the serovar 7) was sequenced. On the basis of
20 sequence comparisons, it was surprisingly found that the oligonucleotides LM1, LM 2, LM3, LM 4, LMF 1, LMF 2, LMR 1 and also sequences complementary thereto lead to highly specific detections in detection methods for *L. monocytogenes*. The preferred probes in this
25 connection are the oligonucleotides LM4, LMR1, LMF1 and LMF2 and the sequences complementary thereto.
30

The invention in particular provides nucleic acid molecules which are characterized in that, with respect
35 to at least 10 successive nucleotides of their nucleotide chain, they

(a) are identical to 10 successive nucleotides of the above nucleic acid molecules (i) to (viii) or

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- (b) match 9 out of 10 successive nucleotides of the above nucleic acid molecules (i) to (viii) or
- (c) match 8 out of 10 successive nucleotides of the above nucleic acid molecules (i) to (viii) or
- 5 (d) are at least 90% homologous to a nucleic acid molecule according to claim 1.

The oligonucleotides of the invention may have a length usual for probes or primers, in particular for a PCR
10 reaction, and further may have a length which can be produced by amplification, in particular by a PCR reaction, and preferably they may be from 10 to 250 bases and in particular from 15 to 30 bases in length.

15 They may be present in single-stranded or double-stranded form.

Thus, suitable microbe-specific oligonucleotides of the invention for detecting *L. monocytogenes* are nucleic
20 acids, preferably from 10 to 250 bases and in particular from 15 to 30 bases in length, which match at least in a 10-base sequence the stated sequences LM 1, LM 2, LM 3, LM 4, LMF 1, LMF 2 and LMR 1 or the sequences complementary thereto. Relatively small
25 deviations (1 to 2 bases) in this 10-base sequence are possible without the specificity stated in each case being lost during amplification and/or hybridization. It is known to the skilled worker that in the case of such relatively small deviations the reaction
30 conditions have to be modified accordingly; cf., for example, T. Maniatis, Molecular Cloning, G. Sambrook & E.F. Fritsch, editors, Cold Spring Harbour [sic] Laboratory Press, 1989.

35 To detect *L. monocytogenes*, nucleic acids, preferably genomic DNA, are first released from cells contained in a sample or bacterial culture to be investigated. It is then possible, by means of nucleic acid hybridization and by using the microbe-specific oligonucleotides

according to the invention as probe, directly to detect
microbe-specific nucleic acid sequences in the sample
to be investigated. Various methods known to the
skilled worker are suitable for this purpose such as,
5 for example, Southern blot or dot blot.

Especially because of the higher sensitivity, however,
an indirect detection method is preferred in which the
sought-after DNA/RNA sequences released as described
10 above are first amplified by means of the
abovementioned methods for amplifying nucleic acids,
preferably PCR.

The primers employed for DNA/RNA amplification using
15 the methods mentioned are the nucleic acid molecules
according to the invention. In this connection,
specific amplified molecules are formed only if
L. monocytogenes DNA/RNA is present. A detection
reaction (following or during the amplification
20 reaction) using the nucleic acid molecules according to
the invention as probes can increase the specificity of
the detection method. In this detection reaction,
oligonucleotides which are not entirely microbe-
specific may likewise be used.

25 An alternative possibility is for the nucleic acid
amplification to be carried out also in the presence of
one or more not entirely specific oligonucleotides so
that possibly DNA/RNA of other microorganisms not to be
30 detected may also be amplified. An amplification method
of this type is usually less specific and should
therefore be safeguarded by a detection reaction
(following or during the amplification reaction) using
one or more of the nucleic acid molecule(s) according
35 to the invention as probe(s).

According to the invention, it is possible to use
various methods in order to detect the amplification
products generated in the indirect methods. These

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include, inter alia, methods known per se such as visualization by means of gel electrophoresis, hybridization of probes to immobilized reaction products [coupled to nylon or nitrocellulose filters (Southern blots) or, for example, to beads or microtiter plates] and hybridization of the reaction products to immobilized probes (for example reverse dot blots or probes coupled to beads or microtiter plates). In addition, it is possible to use methods in which one or more of the nucleic acid molecules according to the invention can, as probes, qualitatively and quantitatively detect specifically forming amplification products during the PCR reaction ("online").

According to the invention, there is a large number of possibilities for the oligonucleotides according to the invention (e.g. probes and primers) to be possibly labeled or modified for the direct or indirect detection methods described. Thus, said oligonucleotides may contain, for example, radioactive, colored, fluorescent or otherwise modified or modifying groups, for example antibodies, antigens, enzymes or other substances with affinity to enzymes or enzyme complexes. Probes and primers may be either naturally occurring or synthetically produced double-stranded or single-stranded DNA or RNA or modified forms of DNA or RNA such as, for example, PNA (in these molecules the sugar units have been exchanged for amino acids or peptides). Individual or a plurality of nucleotides of the probes or primers according to the invention may have been replaced by analogous building blocks (such as, for example, nucleotides which are not naturally present in the target nucleic acid). In particular, up to 20% of at least 10 successive nucleotides of a nucleotide chain, in particular 1 or 2 nucleotides, may have been replaced by analogous building blocks known per se for probes and/or primers.

In the abovementioned indirect detection methods,

detection may also involve an internally labeled amplified molecule. This may be carried out, for example, by incorporating modified nucleoside triphosphates (for example coupled to digoxigenin or fluorescein) during the amplification reaction.

The invention further provides a kit for analytical detection methods, in particular for detecting bacteria of the species *Listeria monocytogenes*, which kit contains one or more nucleic acid molecules according to the invention.

The nucleic acid molecules according to the invention or the appropriate kits may be used in a method for detecting the presence or absence of bacteria of the species *L. monocytogenes* in a sample, said method being preferably a nucleic acid hybridization and/or a nucleic acid amplification, such as a PCR. In this connection, bacteria to be detected can be distinguished from bacteria not to be detected on the basis of differences in the genomic DNA and/or RNA in at least one nucleotide position in the region of one of the nucleic acid molecules according to the invention.

Example

Example 1: Detection of bacteria of the species *L. monocytogenes* using the polymerase chain reaction

DNA was isolated from pure cultures of the bacteria listed in table 1 by means of standard methods. In each case, approx. 10 to 100 ng of these DNA preparations were then introduced to the PCR in the presence of, in each case, 0.4 μ M oligonucleotides LM 1 and LM 2, or LM 3 and LM 2, 200 μ M dNTPs (Boehringer Mannheim), 2.5 mM $MgCl_2$, 16 mM $(NH_4)_2SO_4$, 67 mM Tris/HCl (pH 8.8), 0.01% Tween 20 and 0.03 U/ μ l Taq DNA polymerase (Biomaster). The PCR was carried out in a Perkin Elmer

9600 Thermocycler using the temperature profile listed below:

Initial denaturation	95°C	5 min
35 cycles	94°C	30 sec
	57°C	30 sec
	72°C	30 sec
Final synthesis	72°C	5 min

5

After finishing the PCR reaction, the amplification products were fractionated by means of agarose gel electrophoresis and visualized by ethidium bromide staining. The expected products of 149 bp and 151 bp in length, respectively, were observed only if DNA of strains of the species *L. monocytogenes* was present. The DNA fractionated in the gels was transferred to nylon filters by means of standard methods and hybridized with the 5' digoxigenin-labeled oligonucleotide LM 4 (sequence 4) in order to test the specificity. Hybridization was carried out in 5 × SSC, 2% blocking reagent, 0.1% lauroylsarcosine, 0.02% SDS and 5 pmol/ml probe at 60°C for 4 h. Washing was carried out using 2 × SSC, 0.1% SDS for 2 × 10 min at 60°C. Detection took place using standard methods by means of alkaline phosphatase conjugates (anti-digoxigenin-AP Fab fragment, Boehringer Mannheim) in the presence of 5-bromo-4-chloro-3-indolyl phosphate and 4-nitro blue tetrazolium chloride (Boehringer Mannheim).

25

On the filters, a band was observed only in those cases in which previously a band of 149 bp or 151 bp had been visible on the agarose gel. Thus, the presence of all of the 103 *L. monocytogenes* strains tested was detected by means of PCR and hybridization. In contrast, none of the bacterial strains tested which do not belong to said species were detected by this system.

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Table 1: Results of PCR amplification using the oligonucleotides LM 1 and LM 2 (SEQ ID NO 1 and SEQ ID NO 2) and LM 3 and LM 2 (SEQ ID NO 3 and SEQ ID NO 2), respectively, and, in each case, subsequent hybridization using the oligonucleotide LM 4 (SEQ ID NO 4)

Species	Serovar	Strain	LM1/LM2	LM2/LM3
<i>Listeria welshimeri</i>		SLCC 767	-	-
<i>Listeria welshimeri</i>		SLCC 768	-	-
<i>Listeria welshimeri</i>		SLCC 5877	-	-
<i>Listeria welshimeri</i>		SLCC 5828	-	-
<i>Listeria welshimeri</i>		SLCC 6199	-	-
<i>Listeria welshimeri</i>		DSM 20650	-	-
<i>Listeria seeligeri</i>		SLCC 5921	-	-
<i>Listeria seeligeri</i>		SLCC 7303	-	-
<i>Listeria seeligeri</i>		SLCC 7309	-	-
<i>Listeria seeligeri</i>		SLCC 7329	-	-
<i>Listeria seeligeri</i>		SLCC 3954	-	-
<i>Listeria seeligeri</i>		DSM 20751	-	-
<i>Listeria innocua</i>		SLCC 5326	-	-
<i>Listeria innocua</i>		SLCC 7160	-	-
<i>Listeria innocua</i>		SLCC 7161	-	-
<i>Listeria innocua</i>		SLCC 7167	-	-
<i>Listeria innocua</i>		SLCC 7168	-	-
<i>Listeria innocua</i>		DSM 20649	-	-

<i>Listeria innocua</i>		SLCC 3408	-	-
<i>Listeria innocua</i>		NCTC 10528	-	-
<i>Listeria innocua</i>		SLCC 7139	-	-
<i>Listeria grayi</i>		DSM 20601	-	-
<i>Listeria grayi</i>		DSM 20596	-	-
<i>Listeria grayi</i>		BC 7308	-	-
<i>Listeria ivanovii</i>		DSM 20750	-	-
<i>Listeria ivanovii</i>		SLCC 2028	-	-
<i>Listeria ivanovii</i>		SLCC 2098	-	-
<i>Listeria ivanovii</i>		SLCC 2102	-	-
<i>Listeria ivanovii</i>		SLCC 2379	-	-
<i>Listeria ivanovii</i>		SLCC 4121	-	-
<i>Listeria ivanovii</i>		SLCC 4706	-	-
<i>Listeria ivanovii</i>		SLCC 4770	-	-
<i>Listeria ivanovii</i>		SLCC 5378	-	-
<i>Listeria ivanovii</i>		ATCC 19119	-	-
<i>Listeria ivanovii</i>		ATCC 19111	+	+
<i>L. monocytogenes</i>		ATCC 19112	+	+
<i>L. monocytogenes</i>		ATCC 19113	+	n.d.
<i>L. monocytogenes</i>		ATCC 19114	+	n.d.
<i>L. monocytogenes</i>		ATCC 19115	+	n.d.
<i>L. monocytogenes</i>		ATCC 19116	+	n.d.
<i>L. monocytogenes</i>		ATCC 19117	+	+
<i>L. monocytogenes</i>		ATCC 19118	+	n.d.
<i>L. monocytogenes</i>		SLCC 53	+	+
<i>L. monocytogenes</i>		SLCC 2479	+	+
<i>L. monocytogenes</i>		SLCC 2482	+	n.d.
<i>L. monocytogenes</i>		SLCC 5835	+	+
<i>L. monocytogenes</i>	1 / 2 a	SLCC 4955	+	+
<i>L. monocytogenes</i>	1 / 2 a	SLCC 6204	+	n.d.
<i>L. monocytogenes</i>	1 / 2 a	SLCC 7149	+	+
<i>L. monocytogenes</i>	1 / 2 a	SLCC 7150	+	+
<i>L. monocytogenes</i>	1 / 2 a	SLCC 7153	+	+
<i>L. monocytogenes</i>	1 / 2 a	SLCC 7165	+	n.d.
<i>L. monocytogenes</i>	1 / 2 a	SLCC 7195	+	n.d.
<i>L. monocytogenes</i>	1 / 2 a	SLCC 7196	+	n.d.
<i>L. monocytogenes</i>	1 / 2 a	SLCC 7197	+	n.d.
<i>L. monocytogenes</i>	1 / 2 a	SLCC 7198	+	n.d.
<i>L. monocytogenes</i>	1 / 2 a	SLCC 7973	+	n.d.
<i>L. monocytogenes</i>	1 / 2 a	SLCC 7053	+	n.d.
<i>L. monocytogenes</i>	1 / 2 a	SLCC 7054	+	n.d.
<i>L. monocytogenes</i>	1 / 2 a	SLCC 7055	+	n.d.
<i>L. monocytogenes</i>	1 / 2 b	SLCC 6031	+	n.d.
<i>L. monocytogenes</i>	1 / 2 b	SLCC 7163	+	+
<i>L. monocytogenes</i>	1 / 2 b	SLCC 7151	+	+
<i>L. monocytogenes</i>	1 / 2 b	SLCC 7152	+	n.d.
<i>L. monocytogenes</i>	1 / 2 b	SLCC 7354	+	+
<i>L. monocytogenes</i>	1 / 2 b	SLCC 7367	+	n.d.
<i>L. monocytogenes</i>	1 / 2 b	SLCC 7059	+	n.d.
<i>L. monocytogenes</i>	1 / 2 c	SLCC 4950	+	+
<i>L. monocytogenes</i>	1 / 2 c	SLCC 6793	+	+
<i>L. monocytogenes</i>	1 / 2 c	SLCC 7154	+	+

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<i>L. monocytogenes</i>	1 / 2 c	SLCC 7290	+	n.d.
<i>L. monocytogenes</i>	1 / 2 c	SLCC 7352	+	n.d.
<i>L. monocytogenes</i>	1 / 2 c	SLCC 7355	+	n.d.
<i>L. monocytogenes</i>	3 a	SLCC 4949	+	+
<i>L. monocytogenes</i>	3 a	SLCC 7135	+	n.d.
<i>L. monocytogenes</i>	3 a	SLCC 7179	+	n.d.
<i>L. monocytogenes</i>	3 b	SLCC 2540	+	n.d.
<i>L. monocytogenes</i>	3 b	SLCC 7140	+	n.d.
<i>L. monocytogenes</i>	3 b	SLCC 7381	+	n.d.
<i>L. monocytogenes</i>	3 c	SLCC 2471	+	+
<i>L. monocytogenes</i>	4 a	SLCC 5069	+	+
<i>L. monocytogenes</i>	4 a	SLCC 5070	+	n.d.
<i>L. monocytogenes</i>	4 a / b	SLCC 7083	+	n.d.
<i>L. monocytogenes</i>	4 a / b	SLCC 7065	+	n.d.
<i>L. monocytogenes</i>	4 a / b	SLCC 7069	+	+
<i>L. monocytogenes</i>	4 b	SLCC 4013	+	+
<i>L. monocytogenes</i>	4 b	SLCC 7194	+	+
<i>L. monocytogenes</i>	4 b	SLCC 7356	+	+
<i>L. monocytogenes</i>	4 b	SLCC 7370	+	+
<i>L. monocytogenes</i>	4 b	SLCC 7372	+	+
<i>L. monocytogenes</i>	4 b	SLCC 7373	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7374	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 788	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7056	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7057	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7058	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7060	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7061	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7062	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7063	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7064	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7066	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7067	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7068	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7069	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7070	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7071	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7072	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7073	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7074	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7075	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7076	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7077	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7078	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7079	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7080	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7081	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7082	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7084	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7085	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7086	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7087	+	n.d.

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<i>L. monocytogenes</i>	4 b	SLCC 7088	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7089	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7090	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7091	+	n.d.
<i>L. monocytogenes</i>	4 b	SLCC 7092	+	n.d.
<i>L. monocytogenes</i>	4 c	SLCC 4925	+	n.d.
<i>L. monocytogenes</i>	4 c	SLCC 4954	+	+
<i>L. monocytogenes</i>	4 c	SLCC 6277	+	+
<i>L. monocytogenes</i>	4 c	SLCC 6813	+	+
<i>L. monocytogenes</i>	4 c	SLCC 6821	+	+
<i>L. monocytogenes</i>	4 c	SLCC 6823	+	+
<i>L. monocytogenes</i>	4 d	SLCC 2375	+	n.d.
<i>L. monocytogenes</i>	4 d	SLCC 4926	+	+
<i>L. monocytogenes</i>	4 d	SLCC 4952	+	+
<i>L. monocytogenes</i>	7	SLCC 2622	+	+
<i>Arthrobacter spec.</i>		DSM 312	-	n.d.
<i>Bacillus subtilis</i>		ATCC 6051	-	-
<i>Citrobacter freundii</i>		DSM 30040	-	-
<i>Citrobacter koseri</i>		DSM 4595	-	n.d.
<i>Clostridium bifermentans</i>		DSM 630	-	n.d.
<i>Clostridium sporogenes</i>		IfGB 0303	-	n.d.
<i>Enterobacter agglomerans</i>		IfGB 0202	-	n.d.
<i>Enterobacter cloacae</i>		DSM 30054	-	-
<i>Enterobacter gergoviae</i>		BC 674	-	n.d.
<i>Erwinia carotovora</i>		DSM 30168	-	n.d.
<i>Escherichia coli</i>		DSM 30083	-	n.d.
<i>Hafnia alvei</i>		IfGB 0101	-	n.d.
<i>Klebsiella oxytoca</i>		DSM 5175	-	n.d.
<i>Klebsiella pneumoniae</i>		DSM 2026	-	n.d.
<i>Lactobacillus spec.</i>		IfGB 1401	-	n.d.
<i>Lactob. bifermentans</i>		BC 8463	-	-
<i>Leuconostoc carnosum</i>		DSM 5576	-	n.d.
<i>Leucon. mesenteroides</i>		DSM 2146	-	n.d.
<i>Micrococcus citreus</i>		IfGB 0601	-	-
<i>Micrococcus luteus</i>		DSM 348	-	-
<i>Pediococcus damnosus</i>		BC 505	-	-
<i>Proteus mirabilis</i>		IfGB 51	-	-
<i>Proteus vulgaris</i>		DSM 2041	-	n.d.
<i>Pseudomonas aeruginosa</i>		ATCC 10145	-	n.d.
<i>Pseudomonas fluorescens</i>		IfGB 0301	-	-
<i>Salmonella spec.</i>		BC 2426	-	n.d.
<i>Salmonella typhimurium</i>		BC 2157	-	n.d.
<i>Serratia marcescens</i>		BC 677	-	-
<i>Shigella flexneri</i>		DSM 4782	-	n.d.
<i>Staphylococcus aureus</i>		ATCC 6538	-	-
<i>Streptococcus faecalis</i>		DSM 20380	-	n.d.
<i>Strept. faecalis</i>		DSM 20478	-	n.d.
<i>Strept. diacetylactis</i>		BC 2149	-	-
<i>Strept. thermophilus</i>		DSM 20259	-	n.d.
<i>Yersinia enterocolitica</i>		DSM 4780	-	n.d.

IfGB: Institut für Gärungsgewerbe Berlin
[Institute for Fermentation]

BC: BioteCon Strain Collection

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ATCC: American Type Culture Collection, Rockville,
USA

DSM: Deutsche Sammlung von Mikroorganismen und
Zellkulturen GmbH [German Collection of
Microorganisms and Cell Cultures],

Brunswick, Germany

n.d: not done

Example 2: Online detection of bacteria of the species
5 *L. monocytogenes* using the polymerase chain reaction

DNA was isolated from pure cultures of the strains and
isolates listed in table 2 by means of standard
methods. In each case, approx. 100 fg to 100 ng of said
10 DNA preparations were then introduced to the PCR in the
presence of, in each case, 0.4 μ M oligonucleotide LM 1
and LM 2, 0.2 μ M LMF 1 (label: 3'-fluorescein), LMF 2
(label: 3'-fluorescein) and LMR 1 (label: 5'-LC Red640
(Roche Diagnostics), 3'-phosphate), 200 μ M dNTPs (Roche
15 Diagnostics), 4 mM $MgCl_2$, 3 μ g/ μ l BSA (Roche
Diagnostics), 16 mM $(NH_4)_2SO_4$, 67 mM Tris/HCl (pH 8.8),
0.01% Tween 20 and 0.04 U/ μ l Taq DNA polymerase (HTB).
The PCR was carried out in a Roche Diagnostics GmbH
LightCycler using the temperature profile listed below:

20	Initial denaturation	95°C	2 min
	42 cycles	97°C	0 sec
		59°C	40 sec

During the PCR reaction, fluorescence signals
(detection wavelength 640 nm) were only observed, if
DNA of strains of the species *L. monocytogenes* was
25 present.

Table 2: Results of PCR amplification using the
oligonucleotides LM 1 and LM 2 (SEQ ID NO 1 and SEQ ID
NO 2) and of, in each case, hybridization during the
30 amplification reaction using the oligonucleotides LMF1,
LMF2 and LMR1 (SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7)

Species	Serovar	Strain	LM1/LM2 LMF1/LMF2/LMR1
<i>Bacillus staeroth.</i>		DSM 456	-
<i>Staphylococcus aureus</i>		BC 197	-
<i>Escherichia coli</i> (VTEC)		BC 8318	-
<i>Clostridium perfringens</i>		BC 8799	-
<i>Leuconostoc mesent.</i>		DSM 20241	-
<i>Listeria welshimeri</i>		SLCC 767	-
<i>Listeria welshimeri</i>		SLCC 768	-
<i>Listeria welshimeri</i>		SLCC 5877	-
<i>Listeria seeligeri</i>		SLCC 5921	-
<i>Listeria seeligeri</i>		SLCC 7309	-
<i>Listeria innocua</i>		SLCC 5326	-
<i>Listeria innocua</i>		SLCC 7160	-
<i>Listeria innocua</i>		SLCC 7161	-
<i>Listeria grayi</i>		DSM 20601	-
<i>Listeria ivanovii</i>		SLCC 2028	-
<i>Listeria ivanovii</i>		SLCC 2098	-
<i>Listeria ivanovii</i>		SLCC 2102	-
<i>L. monocytogenes</i>	1 / 2 a	SLCC 4955	+
<i>L. monocytogenes</i>	1 / 2 a	SLCC 6204	+
<i>L. monocytogenes</i>	1 / 2 a	SLCC 7149	+
<i>L. monocytogenes</i>	1 / 2 a	SLCC 7150	+
<i>L. monocytogenes</i>	1 / 2 a	SLCC 7153	+
<i>L. monocytogenes</i>	1 / 2 b	SLCC 7151	+
<i>L. monocytogenes</i>	1 / 2 b	SLCC 7152	+
<i>L. monocytogenes</i>	1 / 2 b	SLCC 7354	+
<i>L. monocytogenes</i>	1 / 2 b	SLCC 7367	+
<i>L. monocytogenes</i>	1 / 2 b	SLCC 7059	+
<i>L. monocytogenes</i>	1 / 2 c	SLCC 6793	+
<i>L. monocytogenes</i>	1 / 2 c	SLCC 7154	+
<i>L. monocytogenes</i>	1 / 2 c	SLCC 7290	+
<i>L. monocytogenes</i>	1 / 2 c	SLCC 7352	+
<i>L. monocytogenes</i>	1 / 2 c	SLCC 7355	+
<i>L. monocytogenes</i>	3 a	SLCC 4949	+
<i>L. monocytogenes</i>	3 a	SLCC 7135	+
<i>L. monocytogenes</i>	3 a	SLCC 7179	+
<i>L. monocytogenes</i>	3 b	SLCC 7140	+
<i>L. monocytogenes</i>	3 b	SLCC 7381	+
<i>L. monocytogenes</i>	3 c	SLCC 2471	+
<i>L. monocytogenes</i>	4 a	SLCC 5069	+
<i>L. monocytogenes</i>	4 a	SLCC 5070	+
<i>L. monocytogenes</i>	4 a / b	SLCC 7083	+
<i>L. monocytogenes</i>	4 a / b	SLCC 7065	+
<i>L. monocytogenes</i>	4 a / b	SLCC 7069	+
<i>L. monocytogenes</i>	4 b	SLCC 4013	+
<i>L. monocytogenes</i>	4 b	SLCC 7194	+
<i>L. monocytogenes</i>	4 b	SLCC 7356	+
<i>L. monocytogenes</i>	4 b	SLCC 7370	+
<i>L. monocytogenes</i>	4 b	SLCC 7372	+

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<i>L. monocytogenes</i>	4 c	SLCC 4925	+
<i>L. monocytogenes</i>	4 c	SLCC 4954	+
<i>L. monocytogenes</i>	4 c	SLCC 6277	+
<i>L. monocytogenes</i>	4 c	SLCC 6813	+
<i>L. monocytogenes</i>	4 c	SLCC 6821	+
<i>L. monocytogenes</i>	4 d	SLCC 2375	+
<i>L. monocytogenes</i>	4 d	SLCC 4926	+
<i>L. monocytogenes</i>	4 d	SLCC 4952	+
<i>L. monocytogenes</i>	7	SLCC 2622	+

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USA

10 DSM: Deutsche Sammlung von Microorganismen und
Zellkulturen GmbH [German Collection of
Microorganisms and Cell Cultures],
Brunswick, Germany

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Patent claims

1. Nucleic acid molecule, **characterized in that**, with respect to at least 10 successive nucleotides of its
5 nucleotide chain, it

(i) is identical to 10 successive nucleotides of the nucleic acid molecules according to a), b), c), d), e), f), g) or h):

10

a) of SEQ ID NO 1 5'-GAA AAA GCA TTT GAA GCC AT-3' or

b) of SEQ ID NO 2 5'-GCA ACT TCC GGC TCA GC-3' or

c) of SEQ ID NO 3 5'-TCG AAA AAG CAT TTG AAG CC-3' or

d) of SEQ ID NO 4 5'-GGT CAG AGT GAA GCT CAT GT-3' or

e) of SEQ ID NO 5 5'-CTI TTC ACA TGA GCT TCA CTC TGA
CCR A-3' or

f) of SEQ ID NO 6 5'-CTT TTT CTT TCA CTG GGT TTC CGA
CAT-3' or

g) of SEQ ID NO 7 5'-GAT GAT TTC TTT TTC TTT CAC TGG ATT
TCC AAT AT-3' or

h) of the sequence complementary in each case to a), b), c), d), e), f) and g); or

15 (ii) matches 9 out of 10 successive nucleotides of the nucleic acid molecules according to (a), (b), (c), (d), (e), (f), (g), or (h) or

(iii) matches 8 out of 10 successive nucleotides of
20 the nucleic acid molecules according to (a), (b), (c), (d), (e), (f), (g), or (h) or

(iv) is at least 90% homologous to a nucleic acid molecule according to (a), (b), (c), (d), (e),
25 (f), (g), or (h).

2. Nucleic acid molecule according to claim 1,

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characterized by a length common for probes or primers, in particular for a PCR reaction, in particular by a length of from 10 to 250 and preferably of from 15 to 30 nucleotides.

5

3. Nucleic acid molecule

- a) of SEQ ID NO 1 5'-GAA AAA GCA TTT GAA GCC AT-3' or
- b) of SEQ ID NO 2 5'-GCA ACT TCC GGC TCA GC-3' or
- c) of SEQ ID NO 3 5'-TCG AAA AAG CAT TTG AAG CC-3' or
- d) of SEQ ID NO 4 5'-GGT CAG AGT GAA GCT CAT GT-3' or
- e) of SEQ ID NO 5 5'-CTI TTC ACA TGA GCT TCA CTC TGA
CCR A-3' or
- f) of SEQ ID NO 6 5'-CTT TTT CTT TCA CTG GGT TTC CGA
CAT-3' or
- g) of SEQ ID NO 7 5'-GAT GAT TTC TTT TTC TTT CAC TGG ATT
TCC AAT AT-3' or

- 10 h) of the sequence complementary in each case to a), b), c), d), e), f) and g).

4. Nucleic acid molecule according to any of the preceding claims, **characterized in that** it is present in single-stranded or double-stranded form.

15

5. Nucleic acid molecule according to any of the preceding claims, **characterized in that** it is present

- (i) as DNA sequence or
- 20 (ii) as RNA sequence corresponding to (i) or
- (iii) as PNA sequence,

25 where the nucleic acid molecule is modified, where appropriate, in a manner known per se for analytical detection methods, in particular for those based on hybridization and/or amplification.

6. Nucleic acid molecule according to any of the

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preceding claims, **characterized in that** up to 20% of at least 10 successive nucleotides of its nucleotide chain, in particular 1 or 2 nucleotides, have been replaced by analogous building blocks known per se for probes and/or primers, in particular by nucleotides not naturally present in bacteria.

7. Nucleic acid molecule according to any of the preceding claims, **characterized in that** the nucleic acid molecule has been modified or labeled by or additionally by having one or more radioactive groups, colored groups, fluorescent groups, groups for immobilization on a solid phase and/or groups for an indirect or direct reaction, in particular for an enzymatic reaction, in particular with the aid of antibodies, antigens, enzymes and/or substances with affinity to enzymes or enzyme complexes, and/or otherwise modifying or modified groups of a nucleic acid-like structure.

8. Kit for analytical detection methods, in particular for detecting bacteria of the species *Listeria monocytogenes*, **characterized by** one or more nucleic acid molecules according to any of the preceding claims.

9. Use of one or more nucleic acid molecules according to any of claims 1 to 7 or of a kit according to claim 8 for detecting the presence or absence of bacteria of the species *Listeria monocytogenes*.

10. Use according to claim 9, **characterized in that** a nucleic acid hybridization and/or a nucleic acid amplification are carried out.

11. Use according to claim 10, **characterized in that** for the nucleic acid amplification a polymerase chain reaction is carried out.

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12. Use according to claim 1 (sic), **characterized in**
that the bacteria to be detected are distinguished from
the bacteria not to be detected on the basis of
differences in the genomic DNA and/or RNA in at least
5 one nucleotide position in the region of one of the
nucleic acid molecules according to claim 3.

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TOT99 T09960

Abstract

The present invention relates to a nucleic acid molecule or nucleic acid molecules and also to a method for the rapid and sensitive detection of bacteria of the pathogenic species *Listeria monocytogenes*. The invention further relates to a test kit or test kits for carrying out the detection methods mentioned.

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)	Attorney Docket Number	2727-133
	First Named Inventor	Pia Scheu
	COMPLETE IF KNOWN	
	Application Number	09 / 786,011
	Filing Date	
	Group Art Unit	
<input type="checkbox"/> Declaration Submitted with Initial Filing	OR	<input checked="" type="checkbox"/> Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)
		Examiner Name

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

"Oligonucleotides, Method and Kit for Detecting Listeria Monocytogenes by Nucleic Acid Amplification and/or Nucleic Acid Hybridization"

the specification of which

(Title of the Invention)

☐ is attached hereto
OR

☒ was filed on (MM/DD/YYYY) 09/02/1999 as United States Application Number or PCT International

Application Number PCT/EP99/06453 and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
DE 198 40 044.6	Germany	09/02/1998	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
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			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	<input type="checkbox"/> Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

(Page 1 of 3)

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(July 1998)

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DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number -	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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OR

☒ Registered practitioner(s) name/registration number listed below

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Gerald Levy	24,419		
Ronald R. Santucci	28,988		
Ronald E. Brown	32,200		

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:		<input type="checkbox"/> A petition has been filed for this unsigned inventor			
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Inventor's Signature				Date	04.26.01
Residence: City	Berlin	State		Country	Germany
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Post Office Address					
City	Berlin	State		ZIP	D-12101
				Country	Germany

☒ Additional inventors are being named on the 1 supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 3 of 3

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Signature

Date

Residence: City

State

Country

Citizenship

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State

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SEQUENCE LISTING

09/786011

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Scheu, Pia
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<120> Oligonucleotides, method and kit for detecting
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